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JUN 2 8 2006
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6/24/03

In the United States Patent and Trademark Office

Parent Appn. Filed: 12/05/2001
Appn. No.: 10/679,305
Applicant: Elizabeth Gay Frayne
Title: "Manipulation of RNA Stability and Protein Synthesis Using Thiophosphate"
Art Unit:1636
Examiner: Nancy T. Vogel

Assistant Commissioner for Patents
Arlington, Virginia, 22313

RE: Response to Office Action Dated 4/20/06

Sir:

In accordance with the office action I have corrected the Abstract to make it one paragraph instead of two. The application is a division of Ser. Nr. 10/007,489 filed 12/05/2001. It is derived from claims 8-13 of the parent application. I have enclosed the revised claims 8-14. The new abstract is also enclosed. I have also enclosed a shortened information disclosure statement with copies of relevant literature. Finally I give my response to the examiner's objections under 103 and 112.

Response to The Claims Rejection Under 103

The present invention does not propose the use of ^{35}S labeled thiophosphate as in Sun et. al. but rather proposes the use of unlabeled thiophosphate as a replacement for normal orthophosphate in growth media. Radio-labeling tracer technology was designed for the sensitive detection of biomolecules in the 10^{-14} mole range using radioactive isotopes. Chemical tests require much higher concentrations of at least 10^{-7} moles. Radio-isotopes are inherently unstable and decay. As such they are generally diluted with the presence of a chemically identical nonradioactive isotope. Radiation can alter an identically labeled molecule by ionization or by ionizing solvent radiolysis especially in aqueous solutions. The radioisotope can also decay to an entirely different compound and in particular ^{35}sulfur decays to ^{35}chlorine. Note that the complete substitution of radiolabeled thiophosphate for phosphate would be anticipated to be lethal to growing cells and destructive to nucleic acids. Note that Sun et. al. uses the label for a exchange purposes in a short incubation period much less than one cell cycle. In contrast unlabeled thiophosphate is not toxic to growing cells and can completely replace phosphate in the growth media for several cell generations. There is no need to use a radioactively labeled molecule as unlabeled thiophosphate readily replaces phosphate.

Sun proposes the use of ^{35}S thiophosphate to label NTP pools to subsequently label nucleic acids. He does not show this and does not imply the use of unlabeled thiophosphate to serve as sole source of phosphate for growing cells. This aspect of my invention is not obvious. Many difficulties in utilizing thiophosphate for growing cells can be envisioned. One is the mechanism of incorporation of thiophosphate into NTP pools in cells. Thiophosphate could be incorporated into ATP during glycolysis or during mitochondrial oxidation. Which route is used could make a difference in the efficiency of incorporation. Furthermore many phosphoryl transfer reactions proceed with retention or conversion of configuration. As several steps are involved in the in vivo synthesis of NTPs from thiophosphate, it is not clear a priori which configuration of nucleotide would be generated. It is known that in vitro polymerases require the Sp -diastereomer of NTP alpha S and proceed with inversion of configuration to generate the Rp configuration. There are also considerations as to the viability of cells and the processing efficiency of RNAs when cells are grown in fully substituted media with such an analogue (see Suh and Waring, (1992) Nucleic Acids Research 20:6303-6309). Generally enzymes are very specific for substrates and do not tolerate such atomic substitutions although they do tolerate isotopic substitutions. There is a big difference between using a radio-labeled tracer molecule to label molecules in cells versus fully substituting and an entire organism's nucleic acids with phosphorothioate linkages.

The present invention shows significant incorporation of thiophosphate since thiophosphate can be used as a sole source of phosphate in growth media even in phosphate depleted cells treated with beta-glycerol phosphate. Cells are viable for several generations and mRNA can be properly processed. Nuclease resistant RNA is obtained from the in vitro analysis of the RNA treated with RNase. Yeast actin mRNA was shown to accumulate several fold as judged by Northern analysis. In addition the intron of the actin mRNA was properly removed.

The present invention provides a method to increase the total amount of mRNA in a cell. This results from the effective in vivo stabilization of RNA molecules with the incorporation of thiophosphate and subsequent generation of phosphorothioate linkages. Stabilization of mRNAs requires an incubation period equal to at least several times the half-life of the typical mRNA in the cell. Owing to the chemical modification of these RNAs they are not only protected in vivo from RNases but also in vitro. To achieve the in vivo stabilization of mRNA requires significant incorporation of thiophosphate into NTP pools and ultimately into nucleic acids. This is not a normal cellular process that is being monitored with a label but rather a gross manipulation of the cell to increase its global mRNA content. Note that while rRNAs can be modified they do not accumulate to the same extent as they are already fairly stable with long half-lives relative to a cell's cyle. This unique property of the invention can be exploited to facilitate the isolation of RNA particularly under conditions where degradation may be a problem.

Response to Claims Rejections 35 USC 112

I have attempted to make the claims more clear and descriptive. Claim 9 was omitted. Claim 14 is now an independent claim rather than a dependent claim

Amended Abtract: Please refer to the substitute abstract enclosed. A marked up version has also been included.. The substitute abstract contains no new matter.

Amended Claims: See Claims Amendment clean version and marked up version enclosed. Claims 8-14 previously numbered 1-7 incorrectly are retained. Claim 9 is omitted. Claim 14 is an independent claim rather than a dependent claim. The changes to the claims do not constitute new matter.

Information Disclosure: I have reduced the number of references in the information disclosure. Enclosed is a new page two. I have also enclosed one new reference for prior art consideration. Copies of these articles are provided.

I wish to declare and make a verified statement that the modified claims and abstract include no new matter as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b) or 1.825 (d).

Please under MPEP 707.07(j) the pro se applicant requests that if the Examiner fines patentable subject matter disclosed in this application, but feels that applicant's present claims are not entirely suitable, the Examiner draft one or more allowable claims for the applicant.

Respectfully submitted,

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